No association between alcohol supplementation and autoantibodies to DNA damage in postmenopausal women in a controlled feeding study

S Mahabir[1], D J Baer[2], L L Johnson[1], K Frenkel, J F Dorgan[3], W Cambell[2], T J Hartman[4], B Clevidence[2], D Albanes[5], J T Judd[2] and P R Taylor[1]

Alcohol consumption is linked to increased breast cancer risk. Since oestrogens increase breast cancer risk, possibly through oxidative damage, and we have shown that alcohol consumption increases serum oestrogens, we tested whether moderate alcohol supplementation increased oxidative DNA damage among healthy postmenopausal women not on hormone replacement therapy in a randomized controlled crossover study. We used serum 5-hydroxymethyl-2-deoxyuridine (5-HMdU) autoantibodies (aAbs) as a marker of oxidative DNA damage. The results showed no evidence for increased or decreased levels of oxidative DNA damage among women who consumed 15 g or 30 g alcohol per day for 8 weeks compared with women in the 0 g alcohol group. We conclude that among healthy women, it is possible that an 8-week trial of moderate alcohol supplementation might be too short to make enough 5-HMdU aAbs to compare differences by alcohol dose. In future studies, a panel of biomarkers for DNA damage should be used. ***European Journal of Cancer Prevention*** **14:427–429 © 2005 Lippincott Williams & Wilkins.**

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[1]Cancer Prevention Studies Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA, [2]Human Nutrition Research Center, Agriculture Research Service, US Department of Agriculture, Beltsville, MD, USA, [3]Population Science Division, Fox Chase Cancer Center, Philadelphia, Pennsylvania, Pennsylvania, USA, [4]Department of Nutrition, Pennsylvania State University, University Park, USA and [5]Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA.

Correspondence to: S Mahabir, Department of Epidemiology, CPB4.3247, UT MD Anderson Cancer Center, Unit 1340, 1155 Pressler Blvd, Houston, TX 77030, USA.
E-mail: smahabir@mdanderson.org

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Introduction

The formation of 5-hydroxymethyl-2-deoxyuridine (5-HMdU), an oxidized DNA base derivative, is one result of oxidative attacks by reactive oxygen species (ROS) (Djuric *et al.*, 2001). It appears that the presence of 5-HMdU in DNA stimulates the production of specific immunoglobulin M-class autoantibodies (aAbs) (Frenkel *et al.*, 1993). Thus, the titres of these antibodies may constitute a marker of oxidative DNA damage and a biological response to that damage (Frenkel *et al.*, 1992, 1993, 1998).

We previously showed that moderate alcohol consumption increased levels of sex hormones (Dorgan *et al.*, 2001), but alcohol has many other biological effects beyond its impact on hormones. In this controlled feeding study, we tested the hypothesis that 8 weeks of moderate (15 g ethanol/day or 30 g ethanol/day) alcohol consumption versus a placebo increases serum 5-HMdU aAbs concentrations in healthy postmenopausal women.

Subjects and methods

The Women's Alcohol Study (WAS) has been described previously (Dorgan *et al.*, 2001; Baer *et al.*, 2002). Briefly, a total of 51 postmenopausal women who were non-smokers and not on hormone replacement therapy (HRT) completed the study and were included in the analysis. The WAS used a crossover design in which each participant rotated through three 8-week controlled dietary periods during which she consumed a daily beverage that contained no alcohol (placebo), 15 g alcohol or 30 g alcohol in random order. Each of the three dietary periods was preceded by a 2- to 5-week washout period where no alcohol was consumed. Alcohol was supplied as 95% ethanol (Everclear; Pharmaco Products Inc, Brookfield, CN, USA) in orange juice (12 ounces (355 ml)). All meals were prepared at the Beltsville Human Nutrition Research Center and the participants ate breakfast and supper at the Center and had carryout lunches on weekdays. On weekends, food and beverages were packaged for consumption at home. The calorie level for each subject was adjusted to maintain constant body weight.

The 5-HMdU assay has been described previously (Frenkel *et al.*, 1998; Wallstrom *et al.*, 2003). Briefly, wells in half of the 96-well plate were coated with 10 µg/ml HMdU-BSA (2 µg of antigen (20 pmol of HMdU)/well (200 µl)), while the other half of the wells were coated with 10 µg/ml M-BSA, sealed with plastic tape, and

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Table 1 Geometric mean 5-HMdU aAbs (A492/μl plasma) for participants on 0 g alcohol and percentage change (Δ) in levels from 0 g to 15 g and 30 g alcohol per day

0 g/day, mean (95% CI)	15 g/day, Δ (95% CI)[a]	30 g/day, Δ (95% CI)[a]	*P* trend[b]
11.7 (9.08–15.1)	1.8% (−5.5–2.1%)	0.8% (−3.0–4.7%)	0.70

[a]Estimates of percentage change are from linear mixed models, including participant as a random effect and alcohol levels as fixed effects treated as two indicator variables.
[b]*P* trend values (two-sided) are from linear models, including participants as a random effect and alcohol levels as a continuous fixed effect with values 0, 15 and 30.

incubated for 3 days at 4°C. Wells were washed three times with TPS and then blocked with 10 μg/ml BSA for 24 h. The antigen-coated wells were incubated (37°C for 2 h) with human sera initially diluted 1 × 104-fold with phosphate-buffered saline (PBS) containing 0.1% BSA. An ELISA plate reader assessed the amount of human aAbs bound to the coated plates at A492/μl.

Serum 5-HMdU aAbs were transformed to the natural log before statistical analysis so that the treatment effects were evaluated as relative changes and error terms were normally distributed. Linear mixed models with a single random intercept reflecting a participant effect were used to estimate changes in 5-HMdU aAbs at 15 g and 30 g of alcohol per day relative to 0 g alcohol per day (i.e. placebo). Models were fitted by treating the alcohol consumption levels as two indicator variables, and as a continuous variable (for dose response). Adjustment for BMI was performed by including it as a fixed effect in the linear mixed models. Carryover effects were analysed by testing treatment order as a fixed effect and testing for an alcohol-by-treatment order interaction in the linear mixed models. Effect modification by race, assignment order, dietary period, age, body mass index (BMI) and years since menopause were assessed by likelihood ratio tests. All tests of statistical significance were two-sided. Statistical analyses were performed using S-PLUS (S-PLUS version 6.1 for Windows, Insightful Corporation, Seattle, Washington, USA, 2002).

Results

The geometric mean concentration of serum 5-HMdU aAs titre (A492/μl plasma) in women who consumed 0 g alcohol per day was 11.7 (95% confidence interval (CI) 9.08–15.1) (Table 1). A consumption of 15 g per day alcohol resulted in a non-significant 1.8% (95% CI −5.5–2.1%) increase compared with 0 g, while 30 g/day alcohol increased 5-HMdU aAbs 0.8% (95% CI −3.0–4.7%). No significant linear trend was seen ($P = 0.70$). We also did not find any effect modification by any of the factors tested.

Discussion

We previously showed that moderate alcohol consumption increased sex hormone levels in postmenopausal women (Dorgan *et al.*, 2001). Because oestrogens have been linked to increased oxidative stress and DNA damage (Cavalieri *et al.*, 2000), and serum markers for oxidative DNA damage have also been shown to increase in women diagnosed with breast cancer (Cavalieri *et al.*, 2000), we sought to test whether this level of alcohol intake would result in increased DNA damage. We considered the 5-HMdU aAbs a superior marker for oxidative DNA damage compared with other markers because it takes into consideration the individual's immune response (Frenkel *et al.*, 1993), it has been shown to significantly increase in individuals consuming high compared with low alcohol estimated from a diet history method (Wallstrom *et al.*, 2003) and it has also been associated with breast cancer risk (Frenkel *et al.*, 1998).

We found no association between alcohol consumption and levels of oxidative DNA damage measured by serum 5-HMdU aAbs, nor did we identify subgroups with elevated levels among these women who were non-smokers and not on HRT. Our study had certain strengths and limitations. The strength of the study is its placebo-controlled, crossover design, which included maintenance of each participant's body weight by controlling for caloric and food composition intake for the duration of the study. This is also the first controlled trial of alcohol supplementation on DNA damage in postmenopausal women. The limitation of the study is that 5-HMdU aAbs is only one of many markers of oxidative DNA damage. While it would likely be better to assess a panel of biomarkers for oxidative DNA damage rather than just one, the development of a single biomarker can be important in risk assessment. In this report, we found neither increased nor decreased levels of 5-HMdU aAbs among postmenopausal women consuming moderate but different amounts of alcohol for 8-week periods in a controlled feeding study.

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